PATENT SPECIFICATION (11) 1 527 605

1 527 605

(21) Application No 34576/75 (22) Filed 20 Aug. 1975

(23) Complete Specification Filed 12 May 1976

(44) Complete Specification Published 4 Oct. 1978

(51) INT. CL.[2] A61K 37/26
(A61K 37/26 45/08)

(52) Index at Acceptance
A5B 248 24Y 26Y 303 30Y 313 31X 31Y
340 341 342 343 344 34Y 38Y 393

(72) Inventors: Shin-ich Iro Hirai
Toshiaki Ikenaga
Tai Matsuzawa

(19)

(54) INSULIN PREPARATION FOR INTRA NASAL ADMINISTRATION

(71) We TAKEDA YAKUHIN KOGYO KABUSHIKI KAISHA, also known as Takeda Chemical Industries Limited, of 27 Doshomachi 2-chome, Higashi-ku, Osaka, Japan a joint stock company of Japan, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

The present invention relates to a novel insulin preparation. More particularly, the present invention relates to an insulin preparation which is clinically suitable for intra nasal administration.

It has hitherto been known that insulin is useful as a medicine for *diabetes mellitus*, for shock therapy in psychiatric disorders, and for the treatment of malnutrition.

Insulin has hitherto been administered solely by injection. Other methods of administration, oral administration, intratracheal administration and rectal administration, have been studied since the discovery of insulin. However, as insulin is a polypeptide comprising about 50 amino acid residues and having a molecular weight of about 6000, it has hitherto been acknowledged that little or no pharmacological effect of insulin is achieved by any of those administration methods other than injection (see "Insulin Monogatari", page 86, published by Iwanamishoten in Japan in 1965).

Long-acting insulin preparations containing zinc or protamine have also been proposed. However, in the case of these preparations a considerable amount of insulin enters into the patient's blood stream all the time, and therefore a dangerous hypoglycemic shock may occur when a patient suffering from *diabetes mellitus* is hungry or asleep. Thus, it is believed that insulin should be injected after food intake to avoid hypoglycemic shock (The Journal of Practical Pharmacy, 25, 505 (1974)). However, it is difficult to administer insulin by means of an injection according to the above dosage schedule on account of the physical pain and the mental suffering involved.

We have made extensive studies in order to find a novel method of administration for insulin as well as an insulin preparation which is free from such disadvantages. At first, we found that when an insulin aqueous solution or suspension having a pH value over 4.7 is contacted with the nasal mucous membrane, a practically useful amount of insulin is not absorbed. Then, we quite unexpectedly found that when an aqueous solution or suspension having a pH value over 4.7 which contains water, insulin and a substance having surface activity (hereinafter the substance is simply abbreviated as a surface-active agent) is contacted with the nasal mucous membrane, insulin is rapidly absorbed from the membrane into the blood stream. This phenomenon was confirmed by the fact that the concentration of plasma glucose is remarkably decreased soon after the intra nasal administration of insulin.

Thus we have found that the intra nasal administration of an aqueous preparation having a pH value over 4.7, which comprises water, insulin and a surface-active agent as defined below establishes a new form of self-medication of insulin and such a medication overcomes the disadvantages of the hitherto known forms of insulin therapy.

We have also found that insulin is advantageously absorbed when the insulin aqueous preparation is contacted with the nasal membrane in the form of a spray.

We have further found that the dose of insulin by intranasal administration required for producing a decrease in plasma glucose which is the same as that obtained by the intramuscular route is from 4 to 6 times greater than the dose of insulin by intramuscular administration.

Furthermore, it is said that insulin in an aqueous preparation may be considerably unstable (Journal of Biological Ch mistry *237*, 3406). We have also made extensive studies in this respect, and have found that an insulin aqueous preparation having a pH value in the range of from over 4.7 to 9.0 which comprises water, insulin and a surface-active agent is free from such instability of insulin.

Therefore, according to the present invention, we provide a new aqueous insulin preparation which permits self-medication with insulin by intranasal administration.

More particularly, we provide an aqueous insulin preparation for intranasal administration having a pH value over 4.7, which comprises water, from 0.1 to 10 weight % of insulin and from 0.1 to 20 weight % of a substance having surface activity which is a nonionic surface-active agent, surfactin, a bile acid salt or a saponin, the aqueous insulin preparation not containing a dialkyl sulphoxide.

Insulin to be employed in the preparations of the present invention may be obtained from natural sources such as mammals (e.g. pigs or cattle), birds or fish, or by chemical reactions according to known processes. Furthermore, the insulin which may be used in the preparations for this invention may include a small amount of harmless impurities. A highly purified insulin, which is called "monocomponent" insulin, can of course also be useful in this invention.

The insulin content in the aqueous preparation of this invention is in the range of from 0.1 to 10 % by weight, and preferably in the range of from 0.2 to 5 % by weight. When the insulin content is less than 0.1 % by weight, the absorption of insulin through the nasal mucous membrane is less. An insulin content of more than 10 % by weight is not economical.

The surface-active agent has been found to promote the absorption of insulin through the nasal mucous membrane into the blood stream.

The surface-active agent may be a natural or synthetic surface-active agent, and is chosen from saponin, bile acid salt, surfactin [Agricultural and Biological Chemistry *33, 1669* (1969)], anionic surface-active agents, amphoteric surface-active agents and nonionic surface-active agents. The surface-active agents employed in the invention may be conveniently chosen from those known *per se*.

Saponin is the so-called sapogenin glucoside, which consists of sapogenin and a sugar such as glucose, galactose, pentose, methylpentose, arabinose or glucuronic acid.

Bile acid salts include, for example, alkali metal salts (e.g. sodium salts) of cholic acid, glycocholic acid, taurocholic acid, cholanic acid, lithocholic acid, desoxycholic acid, chenodesoxycholic acid and dehydrocholic acid.

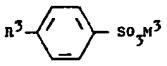
Preferred anionic surface-active agents are exemplified by a carboxylic acid salt of the formula:

$$R^1COOM^1$$

in which R^1 is a hydrocarbon residue having from 7 to 17 carbon atoms, and M^1 is an alkali metal; a sulphonic acid ester salt of the formula:

$$R^2\text{-}O\text{-}SO_3M^2$$

in which R^2 is a hydrocarbon residue having from 8 to 18 carbon atoms, and M^2 is an alkali metal or an organic ammonium ion; and a sulphonic acid salt of the formula:

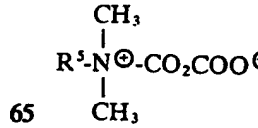


in which R^3 is a hydrocarbon residue having from 8 to 16 carbon atoms and M^3 is an alkali metal.

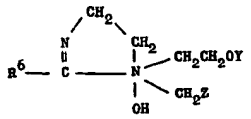
Preferred amphoteric surface-active agents are exemplified by an amino acid-type surface-active agent of the formula:

$$R^4CO\text{-}NH\text{-}A\text{-}COOM^4$$

in which R^4 is a hydrocarbon residue having from 7 to 17 carbon atoms, -NH-A-COO- represents an amino acid residue, and M^4 is an alkali metal; a betaine-type surface-active agent of the formula:



in which R^5 is a hydrocarbon residue having from 8 to 18 carbon atoms; and an imidazole-type surface-active agent of the formula:



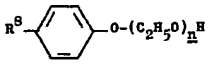
(I)

in which R^6 is a hydrocarbon residue having from 2 to 17 carbon atoms, Y is hydrogen, an alkali metal or a residue represented by $-CH_2COOM^5$ wherein M^5 is hydrogen, an alkali metal or an organic ammonium ion, and Z is a residue represented by $-COOM^5$, $-CH_2-COOM^5$ or $-\underset{\underset{OH}{|}}{CH}-CH_2SO_3M^5$, wherein M^5 is hydrogen, an alkali metal or an organic ammonium ion.

Preferred nonionic surface-active agents are exemplified by polyoxyethylene higher alcohol ethers of the formula:

$$R^7\text{-}O\text{-}(C_2H_5O)_mH$$

in which R^7 is a saturated or unsaturated aliphatic hydrocarbon (e.g. alkyl) radical having from 4 to 18 carbon atoms, and m is an integer from 3 to 60; polyoxyethylene alkyl phenyl ethers of the formula:



in which R^8 is a saturated or unsaturated aliphatic hydrocarbon (e.g. alkyl) radical having from 8 to 10 carbon atoms, and n is an integer from 8 to 70; polyoxyethylene lanolin alcohol ethers of the formula:

$$R^9\text{-}O\text{-}(C_2H_5O)_lH$$

in which R^9 is a steroid alcohol residue or a triterpenoid alcohol residue, and β is an integer from 5 to 40; polyoxyethylene polyoxypropylene higher alcohol ethers of the formula:

$$R^{10}\text{-}(OC_2H_5)_p(OC_3H_7)_qH$$

in which R^{10} is a saturated or unsaturated aliphatic hydrocarbon (e.g. alkyl) radical having from 12 to 18 carbon atoms, p is an integer from 10 to 40, and q is an integer from 1 to 10; polyoxyethylene fatty acid esters of the formula:

$$R^{11}COO(C_2H_5O)_sH$$

in which R^{11} is a saturated or unsaturated aliphatic hydrocarbon (e.g. alkyl) radical having from 7 to 17 carbon atoms, and s is an integer from 5 to 50; and polyvalent alcohol fatty acid esters of the formula:

$$R^{12}OCOR^{13}$$

in which R^{12} is a polyvalent alcohol residue and R^{13} is a hydrocarbon residue having from 7 to 17 carbon atoms. Among the nonionic surface-active agents, those which have a hydrophile/lipophile balance value in the range of from 6 to 20 (i.e. from 6:1 to 20:1) are preferred, and those which have a hydrophile/lipophile balance value in the range of from 8 to 16 (i.e. from 8:1 to 16:1) are particularly preferred. When two or more nonionic surface-active agents are preferably mixed so that the hydrophile/lipophile balance value of the mixture of the surface-active agents is in the above ranges.

In the above formulae, hydrocarbon residues represented by R^1, R^2, R^3, R^4, R^5, R^6 and R^{13} include alkyl radicals which may be straight, branched or cyclic and which may be saturated or unsaturated; aryl radicals such as phenyl or napthyl; and aralkyl radicals such as benxyl or phenethyl. The organic ammonium ion represented by M^2 or M^5 is an organic ammonium ion formed by addition of a proton to an organic amine, preferably a tertiary organic amine such as pyridine, or a trialkyl amine optionally substituted by hydroxy (e.g. triethanol amine). The amino acid residue represented by -NH-A-COO- is formed by eliminating a hydrogen atom from an amino group of an amino acid and at the same time a hydrogen atom from a carboxy group of the same amino acid, the amino acids including, for example glutamic acid, alanine

and β-aminopropionic acid. The steroid alcohol residue and triterpenoid alcohol residue represented by R^9 are a steroid alcohol residue or triterpenoid alcohol residue constituting lanolin. The polyvalent alcohol residue referred to in the definition of R^{12} is a residue formed by eliminating one hydroxy group from a polyvalent alcohol such as glycerin, sorbitol or sucrose.

These surface-active agents may be employed singly or in mixtures of two or more surface-active agents. The content of the surface-active agent or agents relative to the whole preparation is usually in the range of from about 0.1 to about 20 % by weight. A content of less than 0.1% by weight is not enough to promote the absorption of insulin into the blood stream. Although a content of more than 20% by weight also gives a satisfactory effect, it is not practical from an economical point of view.

The pH value of the aqueous insulin preparation of this invention is over 4.7, more particularly in the range of from over 4.7 to 9.0. The aqueous preparation of this invention may be a solution or a suspension. When a highly stabilised solution or suspension is desired, the pH value of the preparation is adjusted in the range of from 6.0 to about 8.5, more preferably in the range of from about 7.0 to about 8.0.

The aqueous insulin preparation of this invention may be produced by mixing the ingredients in any order according to conventional means. Usually, the preparation is produced by dissolving or suspending insulin and one or more surface-active agents in water (more preferably water containing a base (e.g. sodium hydroxide) or an acid (e.g. hydrochloric acid)) and adjusting the pH value of the mixture to the above-mentioned range with a base (e.g. an aqueous sodium hydroxide solution) or an acid (e.g. hydrochloric acid).

Furthermore, if desired, the insulin preparation of this invention may contain other conventional ingredients for aqueous medicaments, e.g. an agent for adjusting the tonicity, an antiseptic agent, a preservative or a buffer.

As regards the manner of administration, the aqueous insulin preparation of this invention may be applied to nasal cavities in the form of a spray by using an atomiser, a nebuliser or a sprayer, whereby the spray of the insulin preparation is contacted with the nasal mucous membrane.

The most prominent merit of such intranasal administration of the insulin preparations of this invention is that it enables all kinds of patients (e.g. human,/even infant) requiring insulin treatment to take insulin by self-medication easily at the desired time without any trouble, pain or other disadvantage.

According to the present invention, the dose of insulin for intrasnasal use in terms of the weight of insulin is about 4 to 6 times that used in intrasmuscular injection.

The invention is illustrated by the following Examples.

Example 1

40 mg of pork insulin (25 units per mg.) are dissolved in 7 ml of a mixture consisting of 100 mg of polyoxyethylene-9-lauryl ether, 1 ml of 0.1N aqueous sodium hydroxide solution, 90 mg of NaCl, 1 ml of pH 7.0 phosphate buffer and water. The solution is adjusted with 0.1N HCl to pH 7.4 and diluted with distilled water to 10 ml. The resulting solution has an insulin potency of 100 units per ml.

Example 2

200 mg of pork insulin (25 units per mg) are dissolved in 7 ml of a mixture consisting of 2 ml of 0.1N hydrochloric acid, 500 mg of saponin and water. The solution is adjusted with 0.1N aqueous sodium hydroxide solution to pH 7.6 and diluted with distilled water to 10 ml. The resulting solution has an insulin potency of 500 units per ml.

Example 3

500 mg of highly purified pork insulin (25 units per mg) are dissolved in 7 ml of a mixture consisting of 1 ml of 0.1N aqueous sodium hydroxide solution, 1 ml of pH 7.0 borate buffer, 300 mg of sodium glycocholate, 160 mg of glycerine and water. The solution is adjusted with 0.1N HCl to pH 7.3 and diluted with distilled water to 10 ml. The resulting solution has an insulin potency of 1250 units per ml.

Example 4

40 mg of pork insulin (25 units per mg) are dissolved in 6 ml of a mixture consisting of 100 mg of polyoxyethylene-9-lauryl ether, 1 ml of 0.1N aqueous sodium hydroxide solution, 500 mg of glucose and water. 1 ml of pH 5.0 acetate buffer is then added to the solution. The resulting suspension is adjusted with 0.1N HCl to pH 5.5 and diluted with distilled water to 10 ml. This suspension has an insulin potency of 100 units per ml.

Example 5

200 mg of pork insulin (25 units per mg) are dissolved in 7 ml of a mixture consisting of 2 ml of 0.1N hydrochloric acid, 500 mg of surfactin and water. The solution is adjusted with 0.1N aqueous sodium hydroxide solution to pH 7.6 and diluted with distilled water to 10 ml. The resulting solution has an insulin potency of 500 units per ml.

Test 1

Effect of Surface-Active Agents of the Nasal Absorption of Insulin in Rats

The insulin preparations for this examination were obtained by the same procedure as in Example 1 except that the surface-active agents were varied.

Male rats (SD-JCL, weighing 200 to 300 g) were anaesthetised with sodium pentobarbital by intraperitoneal injection at a dose of 5 mg per 100 g. 0.1 ml per kilogram of the insulin preparation was administered to the nasal cavity by micropipette and blood samples were drawn from the tail vein at timed intervals after drug administration. Plasma glucose was determined according to the method using *o*-toluidine (Clin. Chem. *8*, 215 (1962)).

The results obtained are shown Table 1, representing the change in plasma glucose following insulin administration. Each value is expressed as the mean of two to nine animals.

For the purpose of comparison, insulin was intra-muscularly injected at 2 units per kilogram.

Amisoft (Trade Mark) CT-12 is cocyl L-glutomate mono-triethanolamine salt. Miranol (Trade Mark) C2M conc. is of the formula (I) as written hereinbefore, wherein R^6 represents $C_{11}H_{23}$, Y represents $-CH_2COONa$ and Z represents -COONa.

Table 1 Effect of Surface-Active Agents on the Nasal Absorption f Insulin in Rats

Dose: 10 Units/Kg (pH=7.4)

	Surface-active agent (Content: 1 %)	Change in Plasma Glucose (%)					
		0	0.5	1	2	3	4 hr.
Control	No surface-active agent	100	102.6	92.7	88.2	92.2	98.8
	Propylene glycol	100	94.2	81.7	80.7	90.1	85.3
	Polyethylene glycol 2000	100	98.7	95.3	93.8	98.3	96.4
	Lecithin	100	99.1	93.1	95.3	91.4	90.7
Saponin	Saponin obtained from tea leaves	100	76.0	43.7	35.0	32.2	41.2
Bile acid salt	Sodium glycocholate	100	72.3	44.5	27.7	38.5	53.0
	Sodium taurocholate	100	80.5	54.8	29.3	33.5	50.3
	Sodium cholate	100	79.6	52.1	38.1	48.1	60.1
Anionic surfactant	Sodium lauryl sulfate	100	68.5	44.3	28.7	45.5	60.1
	Potassium laurate	100	75.4	47.2	30.3	27.2	29.4
	Amisoft CT-12 (Trade Mark)	100	78.5	57.0	39.8	43.6	50.8
Amphoteric surfactant	Miranol C2M conc. (Trade Mark)	100	64.5	44.3	27.3	42.5	54.3
Nonionic surfactant	P.O.E.-9-lauryl ether	100	75.1	39.7	26.0	21.5	31.9
	P.O.E.-10-stearyl ether	100	80.7	60.8	37.0	26.1	24.6
	P.O.E.-10-cetyl ether	100	71.7	39.5	20.2	17.8	15.1
	P.O.E.-5-octyl ether	100	71.0	44.2	37.7	46.1	57.4
	P.O.E.-10-octyl phenyl ether	100	60.7	38.4	31.5	31.4	40.9
	P.O.E.-10-nonyl phenyl ether	100	74.0	50.8	30.2	24.2	34.8
	P.O.E.-24-cholesteryl ether	100	80.1	55.6	40.9	39.90	42.9
	P.O.E.-24-P.O.P.-8-	100	86.8	69.3	53.4	53.8	52.1
	P.O.E.-10-monolaurate	100	76.7	44.4	39.9	47.7	56.5
	Sucrose ester DK-F110	100	86.4	70.0	62.7	64.8	72.0
Surfactin	Surfactin	100	64.8	39.5	31.6	38.1	52.4
	Intramuscular injection 2 units/kg	100	40.0	38.4	28.1	43.2	59.8

Remarks: P.O.E. represents polyoxyethylene
P.O.P. represents polyoxypropylene
* a mixture of mono-, di- and triesters of sucrose with a fatty acid, manufactured by Dai-Ichi Kogyo Seiyaku Co. Ltd.

WHAT WE CLAIM IS:

1. An aqueous insulin preparation for intranasal administration having a pH value over 4.7, which comprises water, from 0.1 to 10 weight % of insulin and from 0.1 to 20 weight % of a substance having surface activity which is a nonionic surface-active agent, an amphoteric surface-active agent, an anionic surface-active agent, surfactin, a bile acid salt or a saponin, the aqueous insulin preparation not containing a dialkyl sulphoxide.

2. An aqueous insulin preparation as claimed in Claim 1, wherein the pH value is in the range of from over 4.7 to 9.0.

3. An aqueous insulin preparation as claimed in Claim 1 or 2, wherein the pH value is in the range of from 6.0 to 8.5.

4. An aque us insulin preparation as claimed in any one of Claims 1 to 3, having a pH value in the range of from 7.0 to 8.0, which comprises water, from 0.2 to 5 weight % insulin and from 0.2 to 10 weight % of the substance having surface activity.

5. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is a nonionic surface-active agent.

6. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is an amphoteric surface-active agent.

7. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is an anionic surface-active agent.

8. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is a bile acid salt.

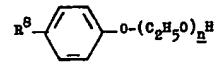
9. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is surfactin.

10. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is a saponin.

11. an aqueous insulin preparation as claimed in Claim 5, wherein the nonionic surface-active agent is a polyoxyethylene higher alcohol ether of the formula:

$$R^7\text{-}O\text{-}(C_2H_5O)_mH$$

in which R^7 is a saturated or unsaturated aliphatic hydrocarbon radical having 4 to 18 carbon atoms, and m is an integer from 3 to 60; a polyoxyethylene alkyl phenyl ether of the formula:



in which R^8 is a saturated or unsaturated aliphatic hydrocarbon radical having 8 to 10 carbon atoms, and n is an integer from 8 to 70; a polyoxyethylene lanolin alcohol ether of the formula:

$$R^9\text{-}O\text{-}(C_2h_5O)_{\mathcal{L}}H$$

in which R^9 is a steroid alcohol residue or a triterpenoid alcohol residue and $\mathcal{L}$ is an integer from 5 to 40; a polyoxyethylene polyoxypropylene higher alcohol ether of the formula

$$R^{10}\text{-}(OC_2H_5)_p(OC_3H_7)_qH$$

in which R^{10} is a saturated or unsaturated aliphatic hydrocarbon radical having 12 to 18 carbon atoms, p is an integer from 10 to 40, and q is an integer from 1 to 10; a polyoxyethylene fatty acid ester of the formula:

$$R^{11}COO(C_2H_5O)_sH$$

in which R^{11} is a saturated or unsaturated aliphatic hydrocarbon radical having 7 to 17 carbon atoms, and s is an integer from 5 to 50; or a polyvalent alcohol fatty acid ester of the formula:

$$R^{12}OCOR^{13}$$

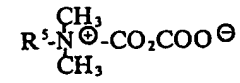
in which R^{12} is a polyvalent alcohol residue and R^{13} is a hydrocarbon residue having 7 to 17 carbon atoms, the hydrophile/lipophile balance value of the nonionic surface-active agent being in the range of from 6 to 20.

12. An aqueous insulin preparation as claimed in Claim 11, wherein the hydrophile/lipophile balance value of the nonionic surface-active agent is in the range of from 8 to 16.

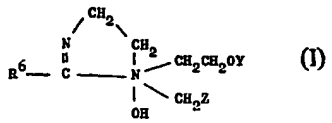
13. An aqueous insulin preparation as claimed in Claim 6, wherein the amphoteric surface-active agent is an amino acid-type surface-active agent of the formula:

$$R^4CO\text{-}NH\text{-}A\text{-}COOM^4$$

in which R^4 is a hydrocarbon residue having 7 to 17 carbon atoms, -NH-A-COO- represents an amino acid residue, and M^4 is an alkali metal; a betaine-type surface-active agent of the formula:



in which R^5 is a hydrocarbon residue having 8 to 18 carbon atoms; or an imidazole-type surface active agent of the formula:



(I)

in which R^6 is a hydrocarbon residue having 2 to 17 carbon atoms, Y is hydrogen, an alkali metal or a residue represented by $-CH_2COOM^5$ wherein M^5 is hydrogen, an alkali metal or an organic ammonium ion, and Z is a residue represented by $COOM^5$, $-CH_2COOM^5$ or $-CH(OH)-CH_2SO_3M^5$, wherein M^5 is hydrogen, an alkali metal or an organic ammonium ion.

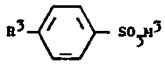
14. An aqueous insulin preparation as claimed in Claim 7, wherein the anionic surface-active agent is a carboxylic acid salt of the formula:

$$R^1COOM^1$$

in which R^1 is a hydrocarbon residue having 7 to 17 carbon atoms, and M^1 is an alkali metal; a sulphonic acid ester salt of the formula:

$$R^2\text{-}O\text{-}SO_3M^2$$

in which R^2 is a hydrocarbon residue having 8 to 18 carbon atoms, and M^2 is an alkali metal or an organic ammonium ion; or a sulphonic acid salt of the formula:



in which R^3 is a hydrocarbon residue having 8 to 16 carbon atoms and M^3 is an alkali metal.

15. an aqueous insulin preparation as claimed in Claim 8, wherein the bile acid salt is an alkali metal salts of cholic acid, glycocholic acid, taurocholic acid, cholanic acid, lithocholic acid, desoxycholic acid, dehydrocholic acid or chenodesoxycholic acid.

16. An aqueous insulin preparation as claimed in Claim 13, wherein -NH-A-COO- is a group formed by eliminating a hydrogen atom from an amino group of an amino acid and at the same time a hydrogen atom from a carboxylic acid of the same samino acid, the amino acid being glutamic acid, alanine or β-aminopropionic acid.

17. An aqueous insulin preparation as claimed in Claim 11, wherein the steroid alcohol residue or triterpenoid alcohol residue is one constituting lanolin, and the polyvalent alcohol residue is a residue formed by eliminating one hydroxy group from glycerin, sorbitol or sucrose.

18. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is saponin obtained from tea leaves.

19. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is sodium glycoholate.

20. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is sodium taurocholate.

21. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is sodium cholate.

22. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is sodium lauryl sulphate.

23. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is potassium laurate.

24. An aqueous insulin preparation as claimed in anyu one of Claims 2 to 4, wherein the substance having surface activity is cocyl L-glutamate mono-triethanolamine salt.

25. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is of the formula (I) as written hereinbefore, wherein R^6 represents $-C_{11}h_{23}$, Y represents $-CH_2COONa$ and Z represents -COONa.

26. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is polyoxyethylene-9-lauryl ether.

27. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is polyoxyethylene-10-stearyl ether.

28. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is polyoxyethylene-10-cetyl ether.

29. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is polyoxyethylene-5-octyl ether.

30. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the

substance having surface activity is polyoxyethylene-10-octyl phenyl ether.

31. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is polyoxyethylene-10-nonyl phenyl ether.

32. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is polyoxyethylene-24-cholesteryl ether.

33. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is polyoxyethylene-24-polyoxypropylene-8-cetyl ether.

34. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the sub-stance having surface activity is polyoxyethylene-10-monolaurate.

35. An aqueous insulin preparation as claimed in any one of Claims 2 to 4, wherein the substance having surface activity is a mixture of mono-, di- and triesters of sucrose with a fatty acid.

36. An aqueous insulin solution as claimed in Claim 1, substantially as herein described with reference to any of the specific Examples.

37. A method for producing an aqueous insulin preparation as claimed in any one of Claims 1 to 36, which comprises mixing in the required proportions water, insulin and a substance having surface activity as defined in any one of Claims 1 to 36, and if necessary adjusting the pH value of the resulting mixture.

38. A method for producing an aqueous insulin preparation as claimed in any one of Claims 1 to 36, which comprises dissolving in water insulin and the substance having surface activity, and adjusting the pH value of the aqueous solution.

39. A method as claimed in Claim 37, substantially as herein described with reference to any of the specific Examples.

40. An aqueous insulin preparation when produced by a method as claimed in any one of Claims 37 to 39.

41. A method for the treatment of diabetes mellitus, which comprises administering to diabetic animals excluding human being an aqueous insulin preparation as claimed in any one of Claims 1 to 36 and 40, the administration being carried out by contacting the preparation with the nasal mucous membrane of the animal.

ELKINGTON & FIFE,
Chartered Patent Agents,
High Holborn House,
52/54 High Holborn,
LONDON WC1V 6SH.

Agents for the Applicants.

Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1978.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.